PATENT SPECIFICATION

NO DRAWINGS

Inventors: FRANTISEK POPPER and SIEGFRIED GOTTFRIED

1,179,787

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Date of filing Complete Specification (under Section 3 (3) of the Patents Act 1949): 14 March, 1968.

Application Date: 22 March, 1967. No. 13394/67.

Application Date: 28 March, 1967. No. 14095/67.

Complete Specification Published: 28 Jan., 1970.

Index at acceptance: —A5 B(31Y, 31X, 311, 312); C3 H3

International Classification:—A 61 k 19/00

COMPLETE SPECIFICATION

Stabilised and Potentiated Therapeutic Enzyme Compositions

We, BIOREX LABORATORIES LIMITED, a British Company, of 47/51 Exmouth Market, Rosebery Avenue, London, E.C.1., do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with new and valuable therapeutic compositions containing enzymes, which can be used for treating various allergic conditions and for diagnosing certain auto-immune diseases. The new compositions can also be used for enhancing protective immunisation, i.e. they can be used prophylactically.

A group of related enzymes, collectively called hyaluronidase (hereinafter called HL) has been known for a number of years. These enzymes, which are found in many tissues and organisms, catalyse the hydrolysis of hyaluronic acid and, in some instances, of chondroitin sulphate A and C, which are major components of the intercellular ground substance.

Although it is recognised that hyaluronidase is not a single enzyme but rather that several such enzymes exist, hyaluronidase has been found to be of great importance therapeutically in the treatment and alleviation of allergic conditions.

In our British Patent Specification No. 1,060,513, there is described and claimed a process for the preparation of an enzyme called glucuronoglycosaminoglycan hyaluronate lyase (hereinafter called GL), this enzyme being found in many tissues and organisms and also catalysing the hydrolysis of hyaluronic acid and of chondroitin sulphate A and C. GL, which appears to be a substantially homogeneous material, is a particularly effective hyaluronidase because of its wide specificity.

We have also found that GL is very useful therapeutically and in our British Patent Specification No. 1,098,957, there are described and claimed compositions for the treatment of alleviations of seasonal and non-seasonal allergic conditions, consisting of an isotonic sterile aqueous solution of highly purified, substantially homogeneous GL.

HL and GL, in common with many other enzymes, tend to lose some or all of their activity upon storage and in our British Patent Specification No. 31066/66 (Serial No. 1120298), we have described and claimed stabilised enzyme compositions containing, in an addition to an enzyme, minor amounts of poly - L - lysine, albumin or protamine.

It has previously been believed that, generally speaking, enzymes do not exert a synergistic activity on one another.

We have now found that a composition containing, as active ingredients, HL and/or GL, in admixture with at least one proteinaceous compound selected from β - glucuronidase, β - N - acetylhexosaminidase, trypsin, chymotrypsin, elastase, ficin, pepsin, streptokinase and lysozyme, exhibits a synergistic action, whereby the therapeutic efficacy of the HL and/or GL, when used for the treatment of alleviation of seasonal or non-seasonal allergic conditions, is potentiated, and that in addition, the HL and/or GL may be stabilised.

Thus, according to the present invention, there is provided a synergistic composition comprising, as active ingredients, HL and/or GL and at least one of the above-mentioned proteinaceous compounds.

The synergistic composition according to the present invention may also contain stabilising materials, such as described in our above-mentioned British Patent Specification No. 31066/66 (Serial No. 1120298), namely,

poly - L - lysine, albumin and/or protamine.

Furthermore, the new composition can be admixed with a solid or liquid pharmaceutical carrier or diluent. Thus, by way of example, the new composition can be made up in the form of a solution in physiological saline.

Although there appears to be no great degree of criticality in the ratios of GL and/or HL to β - glucuronidase, good results have been obtained with a composition containing at least 2500, preferably 5000, Fishman units of β-glucuronidase and at least 500, preferably 5000 I.U. of HL and/or GL in 1 ml. of physiological saline. When β - N - acetylhexosaminidase is present, quantities equivalent to 100 *p* - nitrophenyl units should preferably be present (1 such units is defined as the amount of enzyme liberating 1 mole of *p* - nitrophenol from 3.6 mM *p* - nitrophenyl - N - acetyl - β - glucosamidine in 0.05 M citrate buffer of pH 4.4 in 1 hour at 37°C.). When the new compositions contain trypsin, chymotrypsin, elastase, ficin, pepsin, streptokinase, and/or lysozyme, this is preferably used in an amount of not more than 10% by weight, referred to the weight of HL and/or GL.

The new composition according to the present invention has many useful therapeutic applications, such as in the treatment of various allergic conditions and in the prophylaxis and treatment of certain diseases, including rhinoviral infections, ulcerative colitis, dermatoses and other conditions of immediate or delayed hypersensitivity. Furthermore, when injected into polyps such as nasal, polyps, a marked and rapid regression is observed.

Generally speaking, the new compositions can be administered topically or systemically, for example, by injection, scarification, ingestion, inhalation, or dermally. Furthermore, when the new compositions are in the form of a solution, they can be administered by aerosolisation, i.e. they are sprayed in the form of an aerosol which can, for example, be inhaled or can be forced through the skin under pressure.

The GL and/or HL used in the new composition can be obtained from any convenient source, such as bovine testes. The β-glucuronidase and β-N-acetylhexosaminidase can also be obtained from any convenient source, such as bovine liver, testes and molluscs.

The following Examples are given for the purpose of illustrating the present invention:—

EXAMPLE 1.

β-glucuronidase	5000 Fishman units
GL	5000 I.U.
physiological saline	ad 1 ml.

EXAMPLE 2.

trypsin	25 Anson units
GL	5000 I.U.
physiological saline	ad 1 ml.

EXAMPLE 3.

chymotrypsin	2000 units
GL	5000 I.U.
physiological saline	ad 1 ml.

EXAMPLE 4.

β-glucuronidase	5000 Fishman units
β-N-acetylhexosaminidase	100 units
GL	5000 I.U.
physiological saline	ad 1 ml.

WHAT WE CLAIM IS:—

1. A synergistic composition consisting of hyaluronidase and/or glucuronoglycosaminoglycan hyaluronate lyase and at least one proteinaceous compound selected from β-glucuronidase, β-N-acetylhexosaminidase, typsin, chymotrypsin, elatase, ficin, pepsin, streptokinase and lysozyme.

2. A synergistic composition according to claim 1, wherein there is additionally present at least one member selected from poly-L-lysine, alubumin and protamine.

3. A synergistic composition according to claim 1 or 2, whenever admixed with a solid or liquid pharmaceutical carrier or diluent.

4. A synergistic composition according to claim 3, wherein the carrier or diluent is a physiological saline solution.

5. A synergistic composition according to claim 4, wherein the content of hyaluronidase and/or the lyase is at least 500 I.U. per ml. of saline solution.

6. A synergistic composition according to claim 5, wherein the content of hyaluminidase and/or lyase is at least 500 I.U. per ml. of saline solution.

7. A synergistic composition according to any of the preceding claims, wherein the trypsin, chymotrypsin, elatase, ficin, pepsin, streptokinase and/or lysozyme is used in an amount of not more than 10% by weight, referred to the weight of hyaluronidase and/or the lyase.

8. A synergistic composition according to any of claims 4—7, wherein the content of β-glucuronidase is at least 2500 Fishman units per ml. of saline solution.

9. A synergistic composition according to claim 8, wherein the content of β-glucuronidase is at least 5000 Fishman units per ml. of saline solution.

10. A synergistic composition according to claim 1, substantially as hereinbefore described and exemplified.

11. A method of treating allergic conditions in subjects other than humans, wherein a synergistic composition according to any of claims 1—10 is administered topically or systemically.

12. A method according to claim 11, wherein the composition is administered by injection, scarification, ingestion, inhalation, or dermally.

VENNER, SHIPLEY & CO.,
Chartered Patent Agents,
Rugby Chambers,
2, Rugby Street,
London, W.C.1,
Agents for the Applicants.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1970.
Published by the Patent Office, 25 Southampton Buildings, London W.C.2, from which copies may be obtained.